# The production of deleterious excretions by roots*

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It is our purpose to show in the following paper that the roots of certain higher plants may produce substances which have a deleterious effect. The undue accumulation of these substances is unfavorable for the growth of plants, and hence this study throws some light upon problems of soil conditions and ecological relations.

A number of typically unproductive soils from different parts of the United States have been under study in the laboratories of the Bureau of Soils of the United States Department of Agriculture for several years, and some of their properties are now understood. It has been demonstrated that many soils are unproductive, not because proper nutrients are lacking, but because they contain substances actually deleterious to plant growth.†

An aqueous extract of an unproductive soil, though containing nutrient materials, is often a poorer medium for the growth of plants than distilled water. Wheat seedlings will grow for about three weeks in good distilled water, but various experiments described in the publications just cited show that seedlings grown in the extract of an unproductive soil give a much poorer growth in both top and root, and the plants often die within two weeks.

When such an extract of a poor soil is treated with an insoluble, finely divided solid it loses its toxicity for plant development and gives (as might be expected) a better growth of plants than distilled water. The method usually followed is to add carbon black to the soil extract. The carbon black is stirred or shaken with the soil extract for a few minutes and, at the expiration of a half hour, filtered out. The carbon black contains no nutritive substances. It is practically pure carbon obtained by burning

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† A great deal of evidence on this point has already been presented in Bulletins 28 and 36 of the Bureau of Soils, and a general presentation is given in Farmers' Bulletin 257 of the United States Department of Agriculture.

natural gas and collecting the separated carbon on cool surfaces. It acts by absorbing part of the soluble matter from the soil extract, a power which it possesses to a remarkable extent, by virtue of its enormous surface. The growth of plants in the extract after treatment with carbon black, ferric hydrate or other absorbing agents, is usually greatly increased. The conclusion logically follows that the retarded growth in the original soil extract is due to the presence of some substance or substances actually detrimental to plant development and not to the absence of beneficial nutritive substances.

This experiment is typical of a great number of experiments, employing various unproductive soils. In many cases the growth of plants was greatly improved by diluting the extract with distilled water; in other cases by brief boiling, or by distillation, the toxic properties being found in the distillate. Without dwelling here at length upon the exact data derived from such experiments, it may be said that they agree in showing that the unproductiveness of those soils was due to the presence of substances which exerted a toxic action upon plants. It was also shown that the toxic effect in the soil extracts could be overcome in various ways.

Experiments upon the diminished yields of succeeding crops have given results which indicate that the harmful effect of continuous planting of the same crop may be due to the production of deleterious substances. Many, perhaps a majority, of investigators have assumed that the diminished yield of a second crop is the result of the depletion of the plant nutrients by the first crop. There is now evidence from a number of sources that an important factor in causing diminished yield is the presence of substances detrimental to plant growth. An experiment giving evidence on this point has been described by Livingston ('05) in which wheat was planted in a series of five pot cultures of clean glass sand, simultaneously with five other pot cultures planted in glass sand which had previously grown wheat for twenty-one days. The two series were subjected to the same conditions and growth was measured by the amount of water which the plants transpired. The growth of the plants in the "exhausted" sand was about 45 per cent. of that in fresh sand. In the same experi-

ment the effect of a good absorbing agent was tried. Ferric hydrate was added to five pots of "exhausted" sand in which wheat was planted. This good absorbing agent renovated the sand to such an extent that the growth of wheat in it was only 6 per cent. less than that in the fresh sand. It would be obviously incorrect to assume that the decreased growth of wheat in the sand was due to the depletion of nutrients. Neither can one maintain that the beneficial action of ferric hydrate consisted in supplying nutrients. The only explanation is that the ferric hydrate had a strong absorbent action upon some substance, or substances, which were toxic to the second growth of wheat and which had resulted from the growth of the first crop.

Facts like those cited above give indications that the toxic condition of unproductive and "exhausted" soils may be caused by some substances excreted from the roots of plants. These indications are further supported by the results of all experiments upon the nature of the toxic substances. Up to the present time they all indicate that the toxic substances are organic, and not inorganic, bodies. It has been shown, for example, that the extract from a poor soil may often be benefited by incineration or mere charring of the organic matter present in the residue obtained by evaporating the soil extract, and redissolving it in pure water. When plants are grown in such solutions they show marked improvement over those grown in the original extract. In an experiment described in Bulletin 28 of the Bureau of Soils (p. 29 ff.), 24 wheat plants grown in such a solution showed an increase of 25 per cent. in growth over a control in the original extract. Here there has apparently been a destruction or an alteration of the substances which were previously detrimental to the growth of plants.

Turning to the literature, it will be seen that there are numerous instances in which deleterious excretions have been demonstrated in the lower plants, but in the higher plants there appear to be no definite proofs that such excretions are produced.

Among the earlier botanists there prevailed an idea that roots excreted waste matters. Such ideas were a logical outgrowth of the efforts of their time to correlate the structures and functions of plants with those of animals. Brugmans ('89) alleged that he

had observed small drops to exude during the night from roots of *Viola arvensis* growing in pure sand in a transparent dish. He even stated that he had observed small fragments of material at the extremities of the roots of certain other plants, which he believed to have been exuded from the roots. His observations rest upon what must now be regarded as insufficient evidence and appear to have been made without any of the precautions necessary for a scientific experiment.

The idea that roots excrete waste matters was promulgated by von Humboldt and by de Candolle and by them given prominence in explaining natural plant associations and crop rotations. It is interesting to note that de Candolle ('32, **3**: 1480) stated his belief that the cockscomb and other noxious weeds injured the neighboring plants by some excretion from the roots. It is a matter of common knowledge that de Candolle used his theory of root excretions as a basis for explaining the benefits of crop rotations. He reasoned that the excreta from the roots remaining in the soil would be harmful if the soil were again planted to the same crop; but that, if a different species were planted, it would receive very little harm from the excreta of the previous crop, and even possibly might be benefited by them. Plenck ('94) and Macaire-Prinsep ('32) also endorsed the idea of root excretion. Macaire-Prinsep made an experiment from which he and others drew incorrect conclusions. By separating the roots of a plant into two groups, one of which was placed in a flask containing pure water and the other in a flask containing a solution of sodium chloride or lead acetate, he found that traces of the solute could be detected in the flask originally containing pure water. He believed that the roots in the solution had taken up dissolved matter which had been again actively exuded by the roots. His work was criticised by Braconnot ('39) and Unger ('36), who showed that his results were due to the capillary action of the roots, aided, no doubt, by the siphon which they formed. Walser ('38) and Braconnot ('39) attempted to detect excretions by examining the medium in which plants of the *Papaveraceae* had grown. They believed that it would be possible to demonstrate the presence of opium-like bodies in the medium if the roots produced excretions. Without recognizing the fundamental error of

such an attempt they interpreted the failure of their analysis to mean that no excretions were produced by roots. They deserve credit, however, for showing that Brugmans had entirely misinterpreted the death of the root hairs and the peeling off of the outer layers of the root by assuming that this material was solid excretion from the living root. Nevertheless, several years later Gasparrini ('57) made the absurd statement that he had observed that the root hairs had small lids which opened and emitted secretions.

After the earlier work had been shown to be ill-founded by the investigations of Walser and Braconnot, that line of investigation was given up, and it is only within recent years that any data have been presented on deleterious root excretions.

Newcombe ('02), in describing the growth of roots in closed glass tubes containing water, says that the roots suffered distortion after 12 to 15 hours when the temperature was 23° C. or over. He referred the distortion to a possible lack of oxygen or to the accumulation of root excretions.

Livingston ('05) described an experiment which indicated quite distinctly that toxic substances may arise during growth. The experiment consisted in placing an absorbent agent (carbon black) in a synthetic nutrient solution and measuring the growth of wheat plants in it. The plants from the nutrient solutions containing carbon-black grew 27 per cent. more than the control plants in a solution in which carbon-black was lacking. In the solutions containing ferric hydrate the growth was increased 33 per cent. In another experiment, water redistilled from potassium dichromate and sulphuric acid and from alkaline potassium permanganate was used, the distillates being condensed in a platinum tube. The addition of ferric hydrate to part of the cultures produced an increase of growth (measured by transpiration) amounting to 34 per cent. The conclusion was there drawn that the roots of seedling wheat plants do give off substances which are poisonous to themselves, and that these substances can be removed or corrected by carbon-black or ferric hydrate.

Indirectly there has been gathered some very good evidence upon this point by investigators who have studied the antagonism between different species.

The Woburn Experiment Station ('03) has reported a detailed

study of the antagonism existing between the roots of grass and those of fruit trees. They found that the grass roots had an actively malignant action upon the tree roots which could only be due to some action similar to that of direct poisoning.

Jones and Morse ('03) reported a remarkable case of antagonism between the butternut, *Juglans cinerea*, and cinquefoil, *Potentilla fruticosa*. The *Potentilla* was often killed under and around the butternut trees on a circle of fully twice the diameter of the tree top. *Potentilla* grew vigorously beneath other species of trees without any injury. They found that wherever the *Potentilla* was killed or dying its roots were intertwined and in close relation to those of the butternut trees. The death of the *Potentilla* seemed to be caused by some relation to, or effect from, the roots of the trees.

Another illustration of the antagonistic action of one plant upon another has been given by Jensen ('07), who studied the effect of tree roots upon wheat under experimental conditions. He found that the action of the tree roots had a remarkably depressing effect upon the growth of wheat. The harmful effect was especially marked with certain trees like *Pinus* and *Acer*, while others like *Prunus* were less harmful. The growth of the wheat was most retarded during the summer season when the trees were physiologically active. When, in the autumn, the trees became dormant, the growth of wheat was much improved.

An examination of the literature dealing with the growth of the lower plants shows that different workers have found that the growth of these plants often gives rise to unfavorable conditions in the surrounding medium. Some typical and instructive examples are described by Pfeffer in his *Physiology of plants* (**1**: 512; English translation by Ewart).

Bacteriology has demonstrated quite clearly that deleterious substances are formed during the growth of cultures. Eijkman ('04) has studied the reaction of the waste products of a number of bacteria upon the same and other species. He found that the organisms produced without exception thermolabile substances which inhibited growth. The inhibiting substances were diffusible but could not be filtered through a porcelain filter; they were destroyed when heated to a temperature at which the organisms

were killed. The waste products of a given species were usually more toxic to that and closely related species than to those species more distantly related.

Eijkman's results are confirmed by the recent work of Rahn ('06) on other bacteria. Rahn finds a thermolabile toxic substance which is absorbed by freshly heated clay filters so that a piece of recently heated clay saturated with old bouillon was quickly covered by a growth of organisms. The toxic substance was also destroyed by diffuse light.

The work of Emmerlich and Loew ('99) and other investigators on the action of bacteriolytic ferments and their toxic action on the bacteria of many infectious diseases, involving the preparation of antitoxins and their use in medicine, is of the greatest interest in this connection as showing that the products of bacterial life are poisonous to the living forms of a similar or related species.

Experimenters upon chemotropism have found that the growth of fungus hyphae is not always in the direction of nutrient materials, but they will sometimes grow into toxic substances. Clark ('02) found that the hyphae of *Rhizopus* would grow from a layer of rich nutrient agar into a layer of non-nutrient agar containing $0.005N$ copper sulphate. Fulton ('06) working on the same subject has clearly demonstrated that the hyphae grow in any direction that will carry them out of a region already occupied by numbers of hyphae. He showed that the repelling substance remains in the solution in which the fungi have grown, and that it is not carbon dioxide. The results of the two last-named authors agree in indicating that the fungus hyphae are negatively chemotropic to some substances which they secrete and this negative chemotropism is much greater than any positive chemotropism they may have for nutrients or oxygen.

According to Ferguson ('02) the germination of certain mushroom spores is greatly facilitated when a small bit of living mushroom tissue is included in the culture, but the further development of hyphae from these spores is almost completely inhibited. When the spores which have been germinated are transferred to cultures in which there are no pieces of tissue a continuous development of hyphae takes place. This observation would seem to indicate that the pieces ot living tissue exerted some influence which inhibited

further growth and that its inhibitory effect was first felt by the delicate germ-tubes.

Wehmer ('91, '06) has demonstrated that certain of the fungi do produce a very toxic substance, namely, oxalic acid.

## PLAN OF THE EXPERIMENTS

One general feature of the foregoing work upon excretions has been the indication that the amount of substance excreted is very small. The removal of toxic substances by the use of a small amount of absorbing material or by momentary boiling are evidence that refined methods are necessary in their investigation. The amount of toxic substances present is so small that an ordinary chemical analysis of the soil does not give evidence of them.

The assumption on the part of Braconnot ('39), Walser ('38), Boussingault ('41), and others that these substances existed in sufficient amounts to be detected by ordinary analysis was a fundamental error, which kept other workers from investigating their nature and action. At the same time there is evidence which cannot be overlooked that the plant is sensitive and does respond to the presence of deleterious substances. The following experiments were therefore so planned that the plant itself could be used as an indicator of the excretion of deleterious substances from its roots.

For indicating the presence of a small amount of deleterious material, the rate at which the root of a plant elongates is not entirely satisfactory. Experiments like those of Lilienfeldt ('05) and especially those of Fulton ('06) show that the chemotropic behavior of an organism can be depended upon to indicate the presence of small amounts of deleterious substances. Seedlings of wheat and oats (*Triticum vulgare* and *Avena sativa*) were used as indicators in most of the experiments, since it was found that their roots were chemotropically sensitive to the deleterious substances studied. The wheat seedlings used were germinated in water by a method devised in the laboratory of the Bureau of Soils, and described by Livingston ('06). The seedlings germinated by this method were remarkably uniform, and their roots were straight and free from any adhering particles. The design of the experiments included a study of the behavior of the roots of wheat seedlings in the

presence of excreta from plants of the same and other species. The latter experiments employed seedlings of corn (*Zea Mays*), cowpeas (*Vigna Catjang*) and oats (*Avena sativa*).

For the purpose of making investigations upon the chemotropic behavior of roots it was necessary to employ a medium which was as nearly non-nutrient as possible. In the first experiments clean quartz sand was used as a medium in which to grow the plants. Owing to the difficulty of filling the tubes of small diameter and the necessity (described below) of rotating the cultures, the sand was not suited to the purpose of experimentation. After some preliminary tests, agar agar proved to be a very satisfactory medium to serve as a substratum. The fibers of agar agar were soaked in one or two changes of distilled water at room temperature to remove the slight amount of soluble matter present. The agar agar was then melted by boiling it in distilled water in the ratio of 2 parts of agar to 100 of water. The melted agar was filtered through absorbent cotton and gave a preparation which remained clear when reheated. The agar was poured out and allowed to cool to 30°–35° C. at which temperature it was just beginning to harden. The roots of the seedlings were put in at this time and were firmly held when the agar became set. In agar prepared in this manner the root system developed in a perfectly normal manner. The roots were clean and white, and showed no distortion nor swellings as they do in unfavorable media. The behavior of the roots could be noted at any time through the transparent agar jelly. The glass receptacles in which the seedlings grew were always covered with black paper to exclude light. Since the agar jelly contained about 98 per cent. of water, the plants were able to obtain an ample supply for growth. Small quantities of distilled water were added daily to keep the surface of the agar moist and to prevent the formation of fissures.

The remarkable freedom of the agar from growth of moulds and bacteria showed how free it was from substances which might serve as nutrients. Although the jars containing the agar cultures stood open from 4 to 10 days they were very rarely infested with moulds or bacteria and the few plants that were thus infested were discarded * from the results.

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* *Vide infra* for experiments eliminating bacteria.

## PRELIMINARY EXPERIMENTS

The first experiments were designed to perfect a method of using the plant so that it would serve as an indicator of the deleterious root excretions, if such substances were produced.

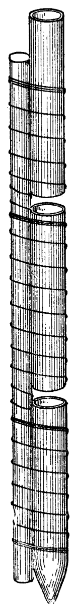

FIG. 1.

Several sorts of perforated tubes of glass and mica were tried; in the end it was found that more satisfactory results were obtained from the use of glass tubes having an internal diameter not greater than 1 cm. The best results were obtained from the use of segmented glass tubes, having small openings between the segments (FIGURE 1). The tubes were made from glass tubing having an internal diameter of 6 or 8 mm. Pieces of tubing 10 cm. long were closed at one end by fusion. Each of these pieces was then cut into three nearly equal lengths. These three segments were then bound to a glass rod, leaving a space of two or three mm. between adjoining segments. They thus formed a straight segmented tube with narrow openings about one-third of the distance from the top and bottom respectively.

The segmented tubes were placed in a vertical position in small glass jars, the fused ends of the tubes resting on the bottom of the jar. Pure non-nutrient agar, which had been washed in three changes of distilled water and melted in distilled water, was poured into the jars until its level reached the tops of the tubes. When the agar had cooled to a temperature between 35° and 38° C., the roots of a wheat seedling three days old were inserted in the open, upper end of each segmented tube.

The design of such an arrangement was to enclose the growing roots in a small space in which the toxic excretions would be confined. At the same time, it provided at intervals small openings through which there would be some diffusion of the toxic products to the exterior. When the growing roots reached one of these regions from which diffusion was taking place, they would have a chance to respond to this unequal distribution of the deleterious substances. Such response would be plainly manifested by the growth of the roots toward regions where deleterious substances were less abundant. In other words, the roots might

curve and grow out of the narrow openings between adjoining segments of the tube.

The first experiment employed a total of 26 roots. The jars containing the tubes and seedlings were wrapped in black paper and stood in a well lighted portion of the laboratory. At the expiration of 6 days, it was found that 14 of the roots had turned from their normal downward course and grown out through the narrow openings into the surrounding agar. The experiment was repeated three times, using a total of 90 roots, 48 of which grew out of the tubes through the narrow openings. It will be noted that this is a response of 53 per cent. of the roots employed.

Since the wheat roots are positively geotropic and possess thereby a natural tendency to grow vertically downward, it must be assumed that there was some definite stimulus acting at the narrow openings of the segmented tubes which caused them to curve and grow out. The roots in these and later experiments showed no distortion nor traumatic curvatures. They always grew straight until reaching the openings through which they passed out into the surrounding medium.

It seems justifiable, therefore, to conclude that their curvature was a response to the presence of some substance or substances to which they were negatively chemotropic. Inside the narrow tube these substances were comparatively concentrated, but at the narrow openings the deleterious substances had partially diffused toward the exterior. When the roots reached the regions from which diffusion had taken place they responded by curving and growing out of the tubes. The same phenomenon of diffusion will explain why the roots did not find the agar in the lower part of the tubes as favorable for growth as that outside the tube.

The following experiment may be cited in further support for the above statements:

Several jars containing melted agar, which had cooled to 36°–38° C., were thickly planted with wheat seedlings. In a few days the roots of the seedlings completely permeated the masses of agar, they were allowed to grow for a week longer and then carefully pulled out, removing as little agar as possible. The agar was melted over a steam bath at a temperature of 70°–80° C., filtered through absorbent cotton and poured into clean jars.

Controls were set up simultaneously which contained freshly prepared agar. Each lot of jars received a set of segmented glass tubes and when the agar had cooled to the proper temperature, wheat seedlings were inserted in the open ends of the segmented tubes. If the foregoing hypothesis regarding the presence and diffusion of toxic waste products be true, one would expect fewer roots to leave the segmented tubes when they were surrounded by a medium which already contained the waste products, because there would be a more equal concentration of them throughout the entire medium. This is precisely what was observed. Of 23 roots which grew in the used agar, only 8 curved and grew out of the tubes. Of 13 control roots, 7 turned and grew out of the tubes.

*Do the roots in the tubes curve because of an insufficient supply of oxygen?* There might be some doubt as to whether part of the response might arise from a deficient supply of oxygen in the small tubes, and the question "Do the roots in the tubes curve because of a possibly insufficient supply of oxygen?" naturally presents itself. The evidence obtained by Bennett ('04) is entirely against such behavior. The author just mentioned made very careful and exhaustive experiments with the roots of land plants, but was unable to find any evidence whatever that direction curvatures could be induced by the one-sided application of such gases as oxygen or carbon dioxide. It was deemed desirable, however, to test the roots of wheat seedlings in the experiments under consideration. A number of segmented glass tubes were set up in jars of freshly prepared agar and a wheat seedling placed in the upper end of each tube. When the agar had become partially solidified, air bubbles were produced by manipulating a glass tube in it. The bubbles were formed at the surface of the segmented tubes and at the level of the narrow openings. When the roots grew downward, they showed the usual curvature at the openings. They did not, however, show any tendency to grow toward the air bubbles, nor to the agar in the vicinity of the air bubbles. Many roots grew directly through air bubbles and passed on without being deflected from their course by the presence of air bubbles. It would be manifestly wrong to base judgment on this question if the roots passed from the agar in the air bubble and remained

there, since the environment would be changed in respects other than air content. One might expect, however, that if an aerotropic tendency were manifested it might be shown by roots turning toward the agar in the vicinity of the air bubbles. Such a tendency was not manifested by roots in this or other experiments where air bubbles were present, hence it follows, that the results reported are really due to a stimulation of the roots by some deleterious substance and not to a deficiency of oxygen.

*The behavior of the roots of oat seedlings in the presence of their own excreta.* — For comparison with the behavior of the roots of wheat seedlings a series of tubes was prepared which held oat plants. The segmented tubes contained and were surrounded by freshly prepared agar. The experiment lasted 6 days. The agar in the tubes became so toxic that 13 of the 39 roots employed were killed. Of the remaining roots, 19 curved and grew out of the tubes. This is a response of 73 per cent. (the roots killed not being considered), and shows that the roots of oat seedlings produced a toxic condition which was quite repellent to themselves. In all cases it was the later roots which were killed. The roots which grew out into the surrounding fresh agar showed no signs of injury.

*Experiments in which malic acid was added to the culture medium.* — Continuous observation of the agar in which plants were grown spoke against the action of the bacteria as a partial factor in producing the results. Nevertheless it was thought profitable to make an experiment in which bacteria were excluded.

After some preliminary experiments which showed that wheat seedlings could tolerate malic acid, a quantity of wheat seedlings were grown in agar which contained 125 parts per million of malic acid. While this amount of acid was not sufficient to inhibit the growth of wheat roots, it reddened litmus paper instantly, and it is safe to assume that bacteria did not develop in such preparations. Part of the segmented tubes contained this agar in which plants had been grown and the others were surrounded by it.

Fifteen roots grew in segmented tubes containing fresh agar and surrounded by used agar. Of this number only four roots grew out into the used agar. Thirty-three roots grew in segmented tubes containing used agar and surrounded by freshly

prepared agar. Sixteen of these roots curved and grew out of the tubes into the freshly prepared agar. The proportion of the response is 56 : 100, which is almost precisely the proportion obtained where no precautions were taken to exclude bacteria. Hence it is believed that the results related in this paper are not induced (although they may be modified) by the action of bacteria.

*Experiments in which the attractive effect of gravity on the roots was neutralized.* — It is evident to anyone familiar with the growth of roots that the positive geotropism, inherent in the wheat roots would hinder their lateral curvature and growth out of the tubes. The responses which have been thus far obtained are therefore the resultant responses to these two stimuli. One might expect that a root which was only feebly stimulated by the presence of the deleterious substances might be more strongly stimulated to grow downward by the attractive force of gravity and hence show no response. The action of gravity cannot be eliminated but it can be neutralized by revolving the plants so that all sides are equally stimulated. Accordingly in the subsequent experiments the preparations containing the seedlings were rotated by attaching them to the arms of a klinostat driven by a small electric motor. The dial which supported the arms was 25 cm. in diameter and revolved once in two minutes. The construction of klinostat and general method of use were similar to the type previously described by Reed ('03).

In all the following experiments the segmented tubes were contained in glass vials 10 cm. long and 2.5 cm. in diameter. The vials were attached to the arms of the klinostat in such a way that they revolved in a plane perpendicular to their long axis. One wheat seedling was placed in each segmented tube and each experiment lasted several days.

Three experiments were performed using freshly prepared agar. Out of a total of 47 roots, 31 curved and grew out of the segmented tubes, a response of 66 per cent. The response in similar tubes which had not been rotated had been 53 per cent.

Summing up the results of these experiments, it appears that the roots of wheat seedlings are repelled by some deleterious substance or substances produced during growth and that a satisfactory means of demonstrating the same is by the use of segmented

glass tubes. Evidence is displayed to show that the curvature of the roots is not due to the action of light, of gravity, of water, nor to a lack of oxygen. Since the medium employed as a soil is as nearly as possible non-nutritive, the question of plant nutrients does not complicate the study. When the effect of gravity is neutralized, a larger proportion of the roots respond to the stimulus produced by the presence of toxic excreta.

## EXPERIMENTS SHOWING THE BEHAVIOR OF WHEAT PLANTS GROWING IN A MEDIUM CONTAINING THE EXCRETA FROM THE ROOTS OF WHEAT OR OTHER PLANTS

With the foregoing results in hand, the next problem was to study the behavior of one plant in the presence of excreta from various other plants. Wheat was selected as the plant to be employed as an indicator, since the roots which it puts out after the short life of the primary root are positively geotropic and sufficiently sensitive for chemotropic reactions.

It was shown in the preliminary experiments that the toxic effects remained in the agar in which the plants had grown. It was also shown that the toxic effects persisted when such agar was melted and used again. In the experiments which are next to be described the effects of root excretions from wheat, corn, cowpeas, and oats were studied. The agar containing their excretions was obtained in each case by planting a large number of seedlings in a dish of soft agar and allowing them to grow for 8 to 15 days, according to the plant employed. In a few days the agar was completely permeated by the roots of the plants, the plants being nourished in the meantime by the reserve materials of the seed. When the agar was to be used for experiment the plants were pulled out, removing as little agar as possible. The agar was then placed in a shallow, covered dish on the steam-bath, stirred and heated to 75–80° C. Continued heating at a high temperature changes or destroys the toxic substance; even at this temperature some of the toxic effect was probably lost. As soon as the agar was completely melted it was filtered through absorbent cotton into the desired receptacles and cooled to room temperature. Distilled water was added to restore that lost by evaporation.

The following series of experiments were so designed that the

used agar was tested against freshly prepared agar, the segmented glass tubes being filled with one and surrounded by the other. The mode of procedure was as follows: Clean segmented tubes were placed in a jar of liquid agar and when it had cooled to the proper temperature a seedling was placed in the mouth of each tube. When the agar had hardened throughout the entire jar, the tubes could be removed without disturbing the seedling or losing any agar from the tubes. The tubes were transferred to vials containing the other sort of agar which had cooled to a temperature between 30° and 35° C. When this agar was completely hardened, the vials were wrapped in black paper and put upon the klinostat. The quantity of agar in which plants had grown was always separated into two portions, one of which was used within a set of tubes and the other portion without another set of tubes. This procedure made each set of results a control on the accompanying results. Thus, the experiments designated under each heading as "a" constitute a check against those designated as "b" and *vice versa*.

WHEAT SUCCEEDING WHEAT.

(*a*) *Wheat plants in tubes containing fresh agar and surrounded by agar in which wheat had been grown.* The used agar was obtained and prepared in the manner outlined above. Five experiments were conducted comprising 89 roots. The details are given in the accompanying table.

| Experiment No. | Roots Capable of Responding. | Roots Which Grew Out of Tubes. | Per Cent. Response. |
|---|---|---|---|
| 1 | 8 | 0 | 0 |
| 2 | 22 | 12 | 55 |
| 3 | 12 | 2 | 16 |
| 4 | 32 | 13 | 40 |
| 5 | 15 | 4 | 26 |

Average for the five samples of agar, 27 per cent.

It will be noted that an average of only 27 per cent. of the roots which grew as far as to the first opening in the tube, turned and grew out into the surrounding agar, in which wheat plants had previously grown, as against 66 per cent. when fresh agar was also used on the outside, as in the experiment already described.

(*b*) *Wheat plants in tubes containing agar in which wheat had*

*been grown and surrounded by fresh agar.* The results of six experiments are presented showing that 62 per cent. of the roots capable of responding grew out of the tubes containing the agar in which a previous wheat crop had grown into the fresh agar surrounding the segmented tube.

| Experiment No. | Roots Capable of Responding. | Roots Which Grew Out of Tubes. | Per Cent. Response. |
|---|---|---|---|
| 1 | 8 | 7 | 87 |
| 2 | 13 | 4 | 31 |
| 3 | 11 | 6 | 55 |
| 4 | 8 | 6 | 75 |
| 5 | 33 | 16 | 48 |
| 6 * | 41 | 30 | 73 |

Average response for the six samples of agar, 62 per cent.

It will be noted that the percentage of diverted roots in the several experiments varies somewhat. This is due to the varying toxicity of the agar derived at different times and from different crops, as shown by the fact that the differences are consistent in the two series, namely, that a high result in series "*a*" is always accompanied by a low result in series "*b*" and *vice versa* for the same sample of used agar.

In the development of roots and tops the plants in series "*b*" excelled. This is probably due to the greater total quantity of fresh agar in that set. As soon as the roots passed out of the segmented tubes the sensitive and absorbing portions were in a medium containing a relatively small amount of toxic excreta. It is obvious from these data that toxic substances remain in the used agar and exert an influence on the succeeding crop. When the roots growing in freshly prepared agar reached the openings in the segmented tubes 27 per cent. of them grew out into the used agar, whereas under the opposite set of conditions 62 per cent. of the roots grew out of the tubes, the proportion of the responses being 44 : 100. This plainly indicates that in the agar which had grown a crop there is some toxic substance, the only source of which could be the excreta from the roots of the previous crop.

WHEAT SUCCEEDING CORN.

The corn used in these experiments was a variety of pop-corn.

* Experiment 6 has no corresponding experiment in table "a" of this series.

When the seedlings had attained a length of 5 cm. they were planted thickly in dishes of soft agar. At the expiration of 10 to 15 days the seedling plants were removed and the agar melted for experiments in the manner previously described.

(*a*) *Wheat plants in tubes containing fresh agar and surrounded by agar in which corn had grown.*— The accompanying table summarizes the results of four experiments. As much as 59 per cent. of the wheat roots capable of response curved out and grew out of the tubes away from their own excreta into the agar containing the excreta of the previous corn crop.

| Experiment No. | Roots Capable of Responding. | Roots Which Grew Out of Tubes. | Per Cent. Response. |
|---|---|---|---|
| 1 | 13 | 7 | 54 |
| 2 | 20 | 10 | 50 |
| 3 | 13 | 9 | 69 |
| 4 | 28 | 17 | 61 |

Average response for the four samples of agar, 59 per cent.

(*b*) *Wheat plants in tubes containing agar in which corn had been grown and surrounded by fresh agar.* — Four experiments were likewise performed in this series. Here 62 per cent. of the roots curved and grew out of the tubes. The details are shown in tabular form.

| Experiment No. | Roots Capable of Responding. | Roots Which Grew Out of Tubes. | Per Cent. Response. |
|---|---|---|---|
| 1 | 24 | 16 | 67 |
| 2 | 32 | 18 | 56 |
| 3 | 39 | 27 | 69 |
| 4 | 9 | 5 | 56 |

Average response of the four samples of agar, 62 per cent.

These results obtained from the use of agar in which pop-corn had previously grown showed that such agar is decidedly less toxic to wheat than the agar in which wheat had grown. In all cases where pop-corn agar was used a high per cent. of roots curved and grew out of the tubes. The figures obtained, 59 and 62 per cent., stand notably near that obtained when only freshly prepared agar was employed, namely 66 per cent. The percentages obtained in series "*a*" and "*b*" are the proportion of 95:100, the difference lying within the limit of experimental error. This

means that the roots behave nearly the same whether the tubes are surrounded by fresh or used agar, and that the toxic effect of the agar in which corn has grown is small, the used agar being, in fact, practically as good as if freshly prepared agar had been used.

WHEAT SUCCEEDING COWPEAS.

The same method of procedure was followed as in the previous experiments.

(*a*) *Wheat plants in tubes containing fresh agar and surrounded by agar in which cowpeas had grown.* — The results of four experiments employing 82 roots are tabulated.

| Experiment No. | Roots Capable of Responding. | Roots Which Grew Out of Tubes. | Per Cent. Response. |
|---|---|---|---|
| 1 | 12 | 8 | 67 |
| 2 | 38 | 21 | 55 |
| 3 | 23 | 9 | 39 |
| 4 | 9 | 6 | 67 |

Average response for the four samples of agar, 57 per cent.

(*b*) *Wheat plants in tubes containing agar in which cowpeas had grown and surrounded by fresh agar.* — The four experiments corresponding to the above employed a total of 96 roots; the details are here given in tabular form.

| Experiment No. | Roots Capable of Responding. | Roots Which Grew Out of Tubes. | Per Cent. Response. |
|---|---|---|---|
| 1 | 20 | 11 | 55 |
| 2 | 31 | 23 | 74 |
| 3 | 26 | 17 | 65 |
| 4 | 19 | 11 | 58 |

Average response for the four samples of agar, 63 per cent.

The results of these two sets of experiments are closely similar to the preceding experiments, which employed agar in which corn had grown. That is to say, it makes little difference so far as this response is concerned, whether the used agar is outside or inside of the segmented tubes. The results in the experiments with cowpeas are in the proportion of 90:100.

This is interpreted to mean that the excreta of the cowpea roots are very slightly toxic to roots of wheat seedlings.

WHEAT SUCCEEDING OATS.

The results of the preceding experiments point quite distinctly to the conclusion that the waste products of some plants are only slightly toxic to wheat seedlings. It seemed desirable to test oats, a more closely related species to wheat than either of the other two tested, and one which in agricultural rotation is a bad crop to precede wheat.

Oat seedlings were allowed to grow for 8 to 10 days in non-nutrient agar, which was then melted for use as in preceding experiments.

(*a*) *Wheat plants in tubes containing fresh agar and surrounded by agar in which oats had grown.* — The accompanying table shows the results of four experiments comprising 87 roots.

| Experiment No. | Roots Capable of Responding. | Roots Which Grew Out of Tubes. | Per Cent. Response. |
|---|---|---|---|
| 1 | 21 | 11 | 52 |
| 2 | 34 | 19 | 56 |
| 3 | 15 | 6 | 40 |
| 4 | 17 | 8 | 47 |

Average for the four samples of agar, 49 per cent.

It will be noticed that only 49 per cent. of the roots grew out of the tubes into the agar in which oats had grown as against 66 per cent. in the case of the fresh agar in the experiments already given.

(*b*) *Wheat plants in tubes containing agar in which oats had grown and surrounded by fresh agar.* The following table gives the results of four experiments which are complements of those given under "*a*."

| Experiment No. | Roots Capable of Responding. | Roots Which Grew Out of Tubes. | Per Cent. Response. |
|---|---|---|---|
| 1 | 24 | 16 | 67 |
| 2 | 21 | 13 | 62 |
| 3 | 45 | 23 | 51 |
| 4 | 19 | 12 | 63 |

Average response for the four samples of agar, 61 per cent.

The results of these experiments show that the agar in which oats had grown was more toxic than that in which corn or cow-peas had grown. When oats agar was used the proportion of

roots which left the tubes in experiments "*a*" and "*b*" was 80:100, showing that there was a sensible difference whether the used agar was within or without the tubes. From these relations we may conclude that the excreta of oats are more toxic to the roots of wheat seedlings than those of corn or cowpeas, a conclusion which is substantiated by the results obtained in crop rotation. If we represent the responses of the roots in the different experiments in the form of percentages, we obtain the following:

Wheat succeeding wheat, 44 per cent.

Wheat succeeding oats, 80 per cent.

Wheat succeeding corn, 95 per cent.

Wheat succeeding cowpeas, 90 per cent.

Summing up the results of these experiments which demonstrate the effect of root excreta from various sources upon wheat roots, it may be noted (1) that the excretions from no other roots were so deleterious to wheat as its own excretions, (2) that the excreta from oats were more harmful than those from the more distantly related plants, cowpeas and corn, (3) that the plants which succeed best in a rotation of crops with wheat, produce excreta which are least harmful to wheat.

## THE RÔLE OF TOXIC EXCRETA IN THE ASSOCIATION AND SUCCESSION OF PLANTS

The production of such toxic excreta as have been demonstrated in this paper throws light upon the problem of association and migration of species and individuals in the vegetable kingdom. The problems of natural association and migration among plants were, as one of us has shown in another publication (Reed '05), among the first studies of ecologists. It has long been known that various physical factors, *e. g.* light, water, etc., often determine the limit of the range of a given species, but it has been repeatedly admitted that they are not sufficient to explain certain important problems of association and succession. The importance and activity of biological factors cannot now be overlooked by any student of ecology. The working of root excreta in causing association and succession are admirably illustrated by the investigations of the Woburn Experiment Station, Jones and Morse, and Jensen, cited in the first part of this paper.

A very apt illustration of the way in which toxic excreta may act in bringing about migration is the case of the "Fairy Ring" fungi. The curiously regular growth of the ring in a continually widening circle may be due to the production of toxic excreta by the growing fungus. The young mycelium grows best on the outside of the ring because it is less affected by the excreta left in the soil within the ring. The common explanation for the development of fairy rings is based upon the assumption that the soil within the ring is so depleted of nutrients that it is unable to support the growth. An investigation of the chemistry of fairy rings by Gilbert ('75) and Lawes ('83) showed such a slight difference between the soil inside and that outside of the ring that this difference cannot explain the entire absence of fungi within the ring. The total nitrogen outside of the rings (average of five rings) was .281 per cent.; inside of the rings it was .247 per cent., a difference of .034 per cent. The average content of carbon outside the rings was 3.30 per cent., inside the rings 2.78 per cent., a difference of .52 per cent. With our present knowledge of the ability of plants to absorb and utilize nutrients, these slight differences are utterly inadequate to explain the entire absence of fungi within the ring. The existence of toxic excreta in the soil would, however, explain it. It is of interest to note that such an explanation of fairy rings was suggested by Way ('47). This investigator admitted "that by far the most scientific and intelligible solution of the question is that which was based upon DeCandolle's theory of the excretions of plants." But on account of objections which appeared insuperable to him he was unable to accept it as a satisfactory explanation.

Another illustration of the possible effect of root excreta in producing associations may be found in the "oak openings." These characteristic grassy tracts existed in the natural oak forests of Ohio, Indiana and southern Michigan before they were modified by man. From some hitherto unexplained cause the forest was apparently unable to advance into these small prairies. In assuming that the grasses produced some substances which were unfavorable to the roots of trees, we have a factor which possibly has importance in the maintenance of such natural societies.

It is quite probable that the excretion of small amounts of deleterious substances is a general phenomenon among all plants.

In view of the very potent effect of the excretions of the plants which have been studied it can only be concluded that the excretions from plants and the accumulation of such excretions in the soil are of the utmost importance in determining such phenomena as association, invasion, and succession.

SUMMARY

1. It has long been known that certain of the lower plants produce substances of an excretory nature which render their environment unsuitable for further growth; but it is only recently that data have been presented to show that the roots of the higher plants may excrete substances which are deleterious to their further growth.

2. The experiments related in this paper show that healthy growing plants excrete from their roots substances which have a deleterious effect upon the growth of the root.

3. The excreta produced by the roots are so small in amount that, up to the present time, they have not been detected by chemical analysis. The chemotropic sensitiveness of the plant does, however, afford a means of detecting and demonstrating experimentally the presence of root excreta.

4. The experiments described in this paper show that, as a rule, the excreta produced by a plant are most toxic to plants of that same species. So far as studied the excreta are more toxic to closely related species than to distantly related ones. Observations in the field indicate that there are specific instances in which the excreta of one species are extremely toxic to other distantly related species.

5. The production of toxic excretions by the roots of the higher plants appears to afford an explanation of some of the important phenomena connected with association, invasion, and succession of plants. It is no less important as an explanation of certain underlying principles in agriculture, chief among which are those of crop rotation and the productivity of the soil.

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